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Prevalence and Risk Factor Analysis of GBV-C/HGV Infection in Prostitutes

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GB virus-C (GBV-C) and Hepatitis G virus (HGV) are variants of a recently cloned virus transmitted parenterally. It is unclear if sexual contact also transmits this virus. In this study, we detected serum GBV-C/HGV RNA in 140 prostitutes by reverse transcription polymerase chain reaction (RT-PCR) using different primers. Thirty (21%) were found with GBV-C RNA by nested PCR although only 22 (73%) had HGV RNA by single round RT-PCR. Both assays had a nearly perfect agreement (kappa value, 0.812). The prevalence of GBV-C RNA in prostitutes was significantly higher than the control group (30/140 vs. 2/40, $P < 0.02$). Multivariate analysis revealed that a frequency of paid sex more than 120 times per month was the only factor significantly associated with positive GBV-C RNA in prostitutes ($P < 0.003$). In summary, prostitutes are a high risk group and reservoir of GBV-C/HGV infection due to high frequency of paid-sex. ***J. Med. Virol. 52:83–85, 1997.***

KEY WORDS: sexual transmission; flavivirus; hepatitis C virus

INTRODUCTION

GB virus-C (GBV-C) is a recently cloned virus [Simons et al., 1995]. Hepatitis G virus (HGV) has more than 90% homology in nucleotide and amino acid sequence with GBV-C [Linnen et al., 1996]. Both viruses are variants of the same virus identified by independent centers, and are members of the flavivirus and distantly related to hepatitis C virus (HCV). GBV-C/HGV infection was found in 1.7% of blood donors and 7% to 39% of patients with various kinds of acute and chronic hepatitis [Simons et al., 1995; Linnen et al., 1996; Aikawa et al., 1996; Fiordalisi et al., 1996]. But its role in fulminant hepatitis remains controversial [Yoshiba et al., 1995; Kao et al., 1996; Mishiro et al., 1996; Kuroki et al., 1996; Salle et al., 1996]. Although its clinical significance is still unknown, GBV-C/HGV is believed to be transmitted parenterally because a higher prevalence was found in patients who gave a history of multiple blood transfusions or intravenous drug abuse [Simons et al., 1995; Linnen et al., 1996; Aikawa et al., 1996].

Sexual contact is the most common route for horizontal transmission of hepatitis B virus (HBV) among adults in Taiwan and sexual contact with prostitutes is the most common mode of HDV infection in this area [Hou et al., 1994; Wu et al., 1990, 1993a]. Although it is generally believed that sexual transmission of HCV is not efficient because of relatively lower concentration of this virus in patients as compared to HBV or HDV, the prevalence of HCV infection among prostitutes was sixfold-higher than among the adult population in this area [Wu et al., 1993b]. It is unclear if sexual contact plays a role in the transmission of GBV-C/HGV.

In this study, serum GBV-C/HGV RNA was detected in a large number of prostitutes by reverse transcription polymerase chain reaction (PCR) using various sets of primers. Questionnaires of potential risk factors associated with viral transmission were completed by prostitutes. Univariate and multivariate analyses were undertaken to identify significant factors associated with GBV-C/HGV infection in prostitutes.

MATERIALS AND METHODS

One-hundred forty randomly-selected female prostitutes in this area were included for testing of serum GBV-C/HGV RNA. These prostitutes had been described previously [Wu et al., 1993a]. Serum samples of these prostitutes were stored in −70°C until use. An interviewer-administered questionnaire was completed by prostitutes with consent. The questionnaire included history of transfusion, dental procedures, injection, or acupuncture with non-disposable needles and syringes, intravenous drug abuse, tattoos, ear-piercing, frequency of paid-sex, gonorrhea, and syphilis. The diagnosis of venereal disease was based on

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TABLE I. Correlation Between the Detection of GBV-C RNA by Nested-PCR and HGV RNA by RT-PCR

		GBV-C RNA by nested PCR			
		First round[a]		Second round[b]	
		+	–	+	–
HGV RNA by RT-PCR[a,b]	+	15	7	22	0
	–	1	117	8	110

[a]Kappa value = 0.757.
[b]Kappa value = 0.812.

clinical and laboratory findings. A physical examination including observations for tattoos or needle-puncture marks was carried out for each subject. Forty (20 HBV carriers and 20 non-carriers) age-matched, non-prostitute females who came for check ups were included as controls. None of either group was positive for antibody to human immunodeficiency virus (HIV). Mann-Whitney U test and chi-square test with Yate's correction were used for the comparison of various parameters between GBV-C RNA positive and negative prostitutes where appropriate. A P value less than 0.05 was considered significant. Univariate and multivariate analyses with logistic regression of factors associated with infection were performed using SAS.

Serum GBV-C/HGV RNA was reverse transcribed using random primers. cDNA was divided for polymerase chain reaction (PCR) using different sets of primers. Nested PCR was undertaken for the detection of GBV-C RNA: primers GB-C-sl and GB-C-al were used in the first round, GB-C.4-sl and GB-C.4-al or GB-C.5-sl and GB-C.5-al were used in the second round as described previously [Simons et al., 1995]. For the detection of HGV RNA, the primers (77F and 211R) were used as reported previously by Linnen et al. [1996]. Strict procedures were followed to avoid false positive results [Kwok and Higuchi, 1989]. Serum alanine aminotransferase (ALT) was measured by a sequential multiautoanalyzer (Technicon SMAC; Technicon Instruments, Tarrytown, NY). The following viral markers were assayed by radioimmunoassay kits: hepatitis B surface antigen (HBsAg) and anti-HDV (Ausria II-125 and Anti-Delta; Abbott Laboratories, North Chicago, IL). Antibody to hepatitis C virus (anti-HCV) was detected by a second-generation enzyme immunoassay (Abbott Laboratories). Anti-HIV was tested by an enzyme immunoassay kit (Rapid Elavia; Diagnostics Pasteur, Mannes-la-Coquette, France).

RESULTS

GBV-C RNA was detected in 16 (11.4%) of 140 prostitutes in the first round of nested PCR using GB-C-sl and GB-C-al as primers. GBV-C RNA was detectable in an additional 14 cases (10%) after the second round using the primers of GB-C.4-sl and GB-C.4-al or GB-C.5-sl and GB-C.5-al as the primers. A total of 30 (21%) prostitutes had detectable GBV-C RNA by nested PCR although only 22 (73%) had HGV RNA by RT-PCR using 77F and 211R as the primers (Table I). Nevertheless, both assays were in nearly perfect agreement with each other (kappa value, 0.812) (Table I). Of the 40 control subjects, only two (5%) (one was a HBV carrier and the other was a non-carrier) showed positive results of GBV-C/HGV RNA. The difference in the prevalence of GBV-C/HGV RNA between prostitutes and controls was statistically significant (30/140 vs. 2/40, $P = 0.017$). While there was no significant difference in age between the two groups (prostitutes 31.9 ± 10.3 years vs. control 34.3 ± 9.5 years, $P = 0.2186$).

TABLE II. Risk Factor Analysis for GBV-C Infection in Prostitutes

Risk factors	GBV-C RNA positive	GBV-C RNA negative
No. of cases	30	110
Age (mean ± S.D., years)[a]	28.0 ± 9.8	33.0 ± 10.3
ALT (mean ± S.D., U/L)	36.7 ± 21.5	39.5 ± 68.4
No. of cases (%) with		
age <28 years[b]	18 (60)	37 (33.6)
positive HBsAg[c]	11 (36.7)	17 (15.5)
positive anti-HCV	4 (13.3)	14 (12.7)
monthly paid-sex >120[d]	22 (73.3)	38 (34.5)
paid-sex >12 months	17 (56.7)	76 (69.1)
history of transfusion	3 (10.0)	4 (3.6)
tattoos	14 (46.7)	48 (43.6)
injection using nondisposable needles	3 (10.0)	13 (11.8)
history of dental procedures	2 (6.7)	23 (20.9)
ear-piercing	18 (60.0)	69 (62.7)
history of acupuncture	3 (10.0)	4 (3.6)
intravenous drug abuse	0 (0)	0 (0)
gonorrhea	14 (46.7)	60 (54.5)
syphilis	3 (10.0)	25 (22.7)

[a]$P < 0.02$; univariate analysis: [b]$P < 0.02$. [c]$P = 0.01$. [d]$P < 0.001$. Multivariate analysis: [d]$P < 0.003$, odds ratio = 4.4; 95% confidence interval 1.7–11.2.

Univariate and multivariate analyses of risk factors associated with GBV-C/HGV RNA were performed among prostitutes. As shown in Table II, the prostitutes with detectable GBV-C/HGV RNA were significantly younger, more often HBV carriers and had a higher frequency of paid-sex as compared with those without in univariate analysis. The remaining factors (such as history of blood transfusion, tattooing, injection with non-disposable needles and syringes, ear-piercing, acupuncture, intravenous drug abuse, dental procedures) were not significant. There were no significant differences in the prevalence rate of HCV infection and serum ALT levels between the two groups. In multivariate analysis, only a frequency of paid sex more than 120 times per month was still associated significantly with positive GBV-C/HGV RNA ($P < 0.003$).

DISCUSSION

There have been few studies to compare the sensitivity of different sets of primers for the detection of GBV-C/HGV RNA. In this study, an almost perfect agreement was found for the detection of GBV-C RNA and HGV RNA using different sets of primers. The primers of 77F and 211R [Linnen et al., 1996] appeared to be superior to GB-C-sl and GB-C-al [Simons et al., 1995] on the basis of the results of the first round of

PCR (22/140 vs. 16/140). However, nested PCR using inner primers of GB-C.5-sl and GB-C.5-al or GB-C.4-sl and GB-C.4-al [Simons et al., 1995] revealed additional positive cases not shown by one round RT-PCR using the primers of 77F and 211R [Linnen et al., 1996]. Our results showed that nested PCR is required for a higher detection rate of GBV-C/HGV RNA.

It is generally believed that GBV-C/HGV is transmitted parenterally [Simons et al., 1995; Linnen et al., 1996; Aikawa et al., 1996]. Because there is no available convenient assay for large scale survey, there has been no report of sexual transmission of this virus. Based on consistent results from different sets of primers, we found a high prevalence rate (21%) of GBV-C/HGV in prostitutes which is significantly higher than in the control group in the same area. This high prevalence rate is comparable to patients with hemophilia, multiply-transfused patients or intravenous drug abuse reported previously [Linnen et al., 1996]. Moreover, a high frequency of paid-sex is significantly associated with GBV-C infection in prostitutes in both uni- and multi-variate analyses. These findings support the view that GBV-C may also be transmitted by sexual contact as HBV, HCV, and HDV are [Hou et al., 1994; Wu et al., 1990, 1993a]. The reason for the association with younger age and HBsAg with GBV-C infection in prostitutes is unclear, however, these factors were no longer significant in multivariate analysis. They may be just dependent factors of high frequency of paid-sex. Although, GBV-C infection is highly prevalent in prostitutes, it did not correlate with serum ALT levels or venereal disease. Most were asymptomatic and had normal or mildly elevated ALT levels. Therefore, the clinical significance of GBV-C/HGV infection is still unknown.

In summary, GBV-C/HGV infection is highly prevalent in prostitutes which may be associated with high frequency of paid-sex.

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